Atty Dkt 2300-0035.22
Client CH-35C
PATENT

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231 on _____

_____ _____
Date Signature

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

LUCIW et al.

Serial No.: 07/138,894 Group Art Unit: 187

Filing Date: 24 December 1987 Examiner: C. Nucker

Title: HUMAN IMMUNODEFICIENCY VIRUS (HIV) NUCLEOTIDE SEQUENCES, RECOMBINANT POLYPEPTIDES, AND APPLICATIONS THEREOF

RULE 132 DECLARATION
OF DR. KATHELYN SUE STEIMER

The Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Dear Sir:

I, KATHELYN SUE STEIMER, HEREBY DECLARE THAT:

1. I am a scientist employed by Chiron Corporation, where I am Director of Cell Biology and Immunology and Project Leader of Chiron's AIDS Vaccine Program.

2. I hold a Ph.D. in Microbiology from the University of Pennsylvania, and have over 18 years experience in

virology. I have authored or co-authored over 40 scientific publications. Attached hereto is my Curriculum Vitae, which lists these publications. I have over 6 years of experience in the immunology of the Human Immunodeficiency Virus (HIV), including the development of immunodiagnostics, and am familiar with the state of the art as of October 1984. I am an ad hoc member of the National Institutes of Health (NIH) Special Study Section on Acquired Immune Deficiency Syndrome. I have authored and been awarded two federally funded (NIH) research grants as principal investigator.

3. I am familiar with the disclosure and data presented in the subject application. (U.S. Application Serial No. 07/138,894, filed 24 December 1987; which is a continuation-in-part of U.S. Application Serial No. 06/773,447, filed 6 November 1985; which is a continuation-in-part of U.S. Serial No. 06/696,534, filed 30 January 1985, now abandoned; which is a continuation-in-part of U.S. Serial No. 06/667,501, filed 31 October 1984, now abandoned.)

4. It is my expert opinion that in October 1984 those of even greater than ordinary skill in the art could not have had any reasonable expectation that a HIV diagnostic using a recombinant antigen would be as effective as the "native" HIV diagnostic then known; i.e., a diagnostic using HIV antigen produced by tissue culture of the virus. The foregoing expert opinion is based on the reasons and facts set forth below.

5. It is my expert opinion that the state of the art in October, 1984 was at best speculative with respect to the general usefulness of recombinant antigens in immunoassays. For a particular virus, there may be factors that prevent development of an effective immunoassay using a recombinant

antigen. These factors can be genuine stumbling blocks which cannot be predicted with any degree of certainty. Not until one has tried and succeeded will it be known that recombinant antigens will be suitable as a replacement for tissue culture-produced antigens.

6. A case which illustrates that producing a successful recombinant immunoassay is not merely a hypothetical problem is shown by Chiron's work with a virus that was better characterized than HIV in October 1984: the Hepatitis A Virus (hereinafter "HAV").

7. Chiron conducted research to develop a recombinant HAV immunoassay. Chiron scientists did not believe that these differences would preclude them from developing a recombinant HAV diagnostic. Upon production of recombinant HAV capsid protein, however, it was unexpectedly discovered that the linear polypeptides that had been recombinantly expressed did not exhibit epitopes that were required to develop an HAV diagnostic. These efforts to produce recombinant HAV useful in an immunoassay were a complete failure. Virtually no antibodies in human serum bound the recombinant capsid antigen, even though it was antigenic in animals; i.e., it elicited antibodies upon injection. Chiron ultimately abandoned its research in developing an assay based on the recombinant HAV capsid antigen. For whatever reason, recombinant antigen-based HAV immunoassays did not work. It was not until HAV capsid was recombinantly expressed that it was discovered that what appeared to be predictable in fact behaved unpredictably.

8. Turning now to HIV, it is important to point out at the outset that there is a basic difference in character between an HIV antigen composition isolated from tissue culture or viral lysate, and an antigen composition produced

by recombinant DNA technology. Recombinantly produced HIV antigen has inherent structural features to distinguish it from HIV antigen isolated from either tissue culture or viral lysate. As explained more fully below, the essential difference is that, due to the nature of HIV, the viral lysate antigen composition is necessarily a heterogeneous composition which exhibits variations in amino acid sequence; the recombinant antigen composition necessarily has a homogeneous amino acid composition. This, of course, could be expected to affect the relative immunological properties of the two compositions. The following articles are attached hereto and will be referred to below:

Meyerhans et al., Cell 58:910-910 (1989)
Fenyö et al., AIDS 3 (Suppl. 1):S5-S12 (1989)
Wain-Hobson, AIDS 3 (Suppl. 1):S13-S18 (1989)

9. HIV is a retrovirus that replicates via RNA-dependent reverse transcriptase encoded in the pol domain of HIV. It is well known that reverse transcriptases do not make authentic copies during the replication cycle of the virus, and the misincorporation rate of HIV reverse transcriptase has been estimated to be about 10^{-4} per base, which is equivalent to one nucleotide change per genome per replication cycle. See Meyerhans et al., at 901, col. 1. It has therefore been statistically concluded that no two HIV proviruses (the DNA replicate of the viral genome made in vivo), are identical. See Wain-Hobson at S13, col. 1. Thus, a "single" viral isolate is actually heterogeneous and consists of many variants. Fenyö et al., at S6, col. 1.. It is also well-known that the env domain of HIV has "hypervariable" regions that can differ greatly from isolate to isolate.

10. The papers cited in paragraph 8 supra show specific examples of the heterogeneous (polymorphic) nature

of HIV. For example Meyerhans et al., Fig. 5 at 906, compares the amino acid sequence of a very small protein, tat, encoded by in the HIV genome. Three different groupings of heterogeneous amino acid sequences found in samples taken from cell culture (peripheral blood mononuclear cells or "PBMC") are shown as groupings L1-L3. They are compared to heterogeneous groupings of tat amino acid sequences found in samples of viral isolates taken directly from a patient (V1-V3). As can be seen, sample L1 had at least seven different "species" of tat protein; sample L2 contained at least eight different proteins; and sample L3 was a heterogeneous mixture also of at least eight different proteins. Wain-Hobson reiterates this same data in Figure 2, with the addition of similar data for the env domain shown in Figure 1 at page S14. Wain-Hobson also observed sequence changes in the gag domain. Wain-Hobson at S15, col. 1.

11. The sequence heterogeneity or polymorphism described in paragraph 10 has also been observed with the HIV isolates known in 1984. For example, Institut Pasteur has described the isolation of LAV-1. See, e.g., Barre-Sinoussi et al., (1983) Science 220:868-871 (1983) (Ref. Desig. AR-1 on PTO 1449 submitted 2/21/90); U.S. Patent No. 4,708,818 (to Montaignier et al.) at col. 2, line 52 (Ref. Desig. AB-1 on PTO 1449 submitted 1/19/90); EP-A-0 138 667 at 24, line 9 (Ref. Desig. AM-1 on PTO 1449 submitted 2/21/90); WO 86/02383 at 9, line 9 (note also reference to clone J19 at 8, line 28) (Ref. Desig. AL-1 on PTO 1449 submitted herewith). The National Institutes of Health researchers disclosed, in their papers and patent application, HTLV-III$_B$. See, e.g., Gallo, et al. Science 224:500-504 (4 May 1984) (Ref. Desig. AT-1 on PTO 1449 submitted 2/21/90); U.S. Patent No. 4,520,113, col. 1, line 23 (Ref. Desig. AA-1 on PTO 1449 submitted herewith); WO 85/04903 at 4, lines 31 and 36 (Ref. Desig. AO-1 on PTO 1449

submitted 2/21/90); EP-A-0 173 529 at 6, line 7 (Ref. Desig. AL-2 on PTO 1449 submitted 2/21/90) (CRL 8543 is the same isolate as in 85/04903 at 4, line 31; note also clones BH10, BH5 and BH8 at 6, lines 7-8). Wain-Hobson noted polymorphism for both the LAV-1 and HTLV-III_B isolates, as well as in the clones specifically disclosed in the above papers and patent specifications. Wain-Hobson at S13, sentence bridging col. 1 and 2. The applicants herein further noted the polymorphic nature of ARV (the isolate in Levy et al.), by observing that different ARV isolates had differences in their restriction maps. Specification, Example 6 at 18-19.

12. Based upon the foregoing, it is clear that an immunoassay or polypeptide composition comprised of any particular HIV antigen, including the env antigen, isolated from native virus, grown in culture or otherwise, will necessarily constitute a mixture of similar, but not identical, proteins which are heterogeneous in amino acid sequence. Some of these amino acid changes will affect the immunological properties of the proteins, i.e., amino acid changes can destroy, change, or create an epitope. In October 1984 it could have been reasonable to expect that the sensitivity of the conventional lysate-based assays was not attributable, at least in part, to the heterogeneity in antigen composition; since no two HIV isolates were identical, it may have been important in screening blood samples from a diversity of human subjects to have some variation in antigen sequences to improve the chances of binding as many anti-HIV antibodies as possible.

13. An antigen composition made up of recombinant HIV env polypeptide is inherently homogeneous in amino acid sequence relative to the lysate-derived HIV antigens, such as those described in Gallo et al., U.S. Patent No. 4,520,113, and Montaignier et al., U.S. Patent No.

4,708,818. A recombinant DNA vector is expressed in the host cell via transcription with the host cell's DNA-dependent RNA polymerase. This transcription is not subject to the high degree of error found with HIV reverse transcriptase. Thus, a single DNA sequence is cloned and expressed to produce an essentially homogeneous composition of recombinant polypeptide. For example when an env polypeptide is made by a recombinant DNA process, the resulting antigen composition is homogeneous in amino acid sequence relative to the lysate derived compositions. In other words, the recombinant env antigen composition will lack the amino acid sequence heterogeneity of the virus-derived antigen composition.

14. In conclusion, therefore, it is my expert opinion that prior to October 1984, it would not have been predictable that factors, such as the above homogeneous nature of a recombinant env antigen composition, would not have caused a failure in the development of a recombinant HIV immunoassay. Indeed, it was not unlikely that a recombinant HIV immunoassay, particularly one based on the env polypeptide, would have been a failure similar to HAV.

15. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 9/14/90 Kathelyn Sue Steimer

KATHELYN SUE STEIMER